

AMENDMENT TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Currently Amended) A method of identifying, screening, characterizing or designing a chemical entity which mimics or binds to a FIH (Factor Inhibiting HIF (Hypoxia Inducible Factor)) polypeptide comprising SEQ ID NO:21, or a fragment or mutant thereof that adopts a similar 3-dimensional structure as described by the structural factors or structural coordinates shown in Table 3 and wherein the fragment or mutant retains asparaginyl hydroxylase activity, which method comprises comparing a structural model of said FIH with a structural model for said chemical entity, wherein said structural model of said FIH is derived from structural factors or structural coordinates ~~determined by subjecting to X-ray diffraction measurements a crystal comprising FIH~~ shown in Table 3.

2. (Canceled).

3. (Canceled).

4. (Currently Amended) A method according to claim 1, wherein said chemical entity binds to the FIH.

5. (Currently Amended) A method according to claim 1, wherein said chemical entity is selected to inhibit the asparaginyl hydroxylase activity of the FIH.

6. (Currently Amended) A method according to claim 1, further comprising contacting said chemical entity with a HIF polypeptide comprising SEQ ID NO:24 or 25 or a fragment thereof or a homologue of either variant having at least 90% identity thereof wherein said fragment or variant comprises the asparagine residue therein and retains the capacity to bind to FIH, incorporating asparagine-803 and with the FIH polypeptide or a homologue fragment or mutant thereof which maintains the asparaginyl hydroxylase activity of FIH and monitoring for hydroxylation of said asparagine residue 803.

7. (Withdrawn) A chemical entity identified by a method according to claim 1, wherein said chemical entity inhibits the asparaginyl hydroxylase activity of FIH.

8. (Withdrawn) A chemical entity according to claim 7 wherein said chemical entity inhibits hydroxylation of the asparagine position 803 of HIF by FIH.

9. (Withdrawn) A chemical entity according to claim 7 wherein said chemical entity inhibits dimerisation of FIH.

10. (Withdrawn) A chemical entity according to claim 9 wherein said chemical entity binds to residues that form the dimerisation interface of FIH, selected from residues 330 to 346 of FIH.

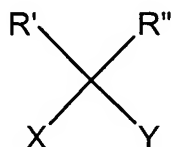
11. (Withdrawn) A chemical entity according to claim 7 wherein said chemical entity binds to iron, or prevents Fe(II) binding to FIH.

12. (Withdrawn) A chemical entity according to claim 11, wherein said chemical entity is a compound selected from a thiol, alcohol, phenol, carboxylate, hydroxamate, imidazole or other heterocyclic compound, that binds to iron.

13. (Withdrawn) A chemical entity according to claim 7 wherein said chemical entity disrupts 2-oxoglutarate binding to FIH.

14. (Withdrawn) A chemical entity according to claim 13, wherein said chemical entity is R-entimer of N-oxaloylalanine, procollagen prolyl-hydroxylase and a PHD isozyme.

15. (Withdrawn) A chemical entity according to claim 13, wherein said chemical entity is a compound of the formula

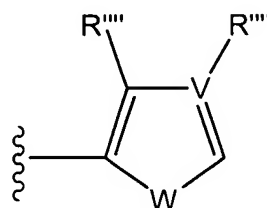
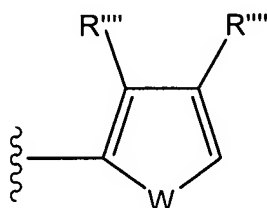
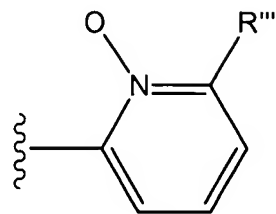
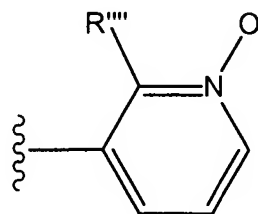
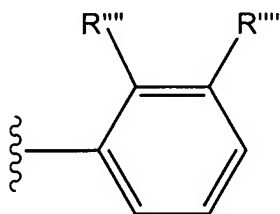


(I)

wherein each of R' and R'', which may be the same or different, is H, F or C₁ to C₃ alkyl or substituted alkyl, CH₂OH, CH₂CO₂H or CONH₂, X is COOH, SOOH, or CONHH or an ester thereof, or other group which forms a favourable interaction with one or more of the side chains of Lys-214, Thr-196 and Tyr-145,

Y is - (CR'''R''')_nZ, where Z is

-NR^{'''}COCOOH, -NR^{'''}CSCOOH, -NR^{'''}COCOSH, -CHSR^{'''}CONR^{'''}R^{'''},
-CHOR^{'''}CONR^{'''}OR^{'''}, -CHSR^{'''}CONR^{'''}OR^{'''} or -CHOR^{'''}CONR^{'''}NR^{'''}OR^{'''}, wherein each R^{'''},
which may be the same or different, is H, alkyl, OH or O-alkyl, n is 0 to 3, or



wherein R^{'''} is OH, OR^{'''} or NHCOR^{'''}, and W is S, NH, or O;

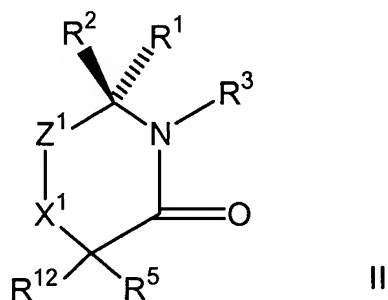
16. (Withdrawn) A chemical entity according to claim 13 wherein said chemical entity interferes with the interactions at residues 214, 196 and 145 of FIH, or which interrupts the interactions of 2OG with residues 281, 186, 188, 207 or 196 of FIH.

17. (Withdrawn) A chemical entity according to claim 16 wherein said chemical entity interrupts binding of FIH for Asn 803 of HIF, preferably, by interfering with binding of HIF at residues 102, 239 or 238 of HIF.

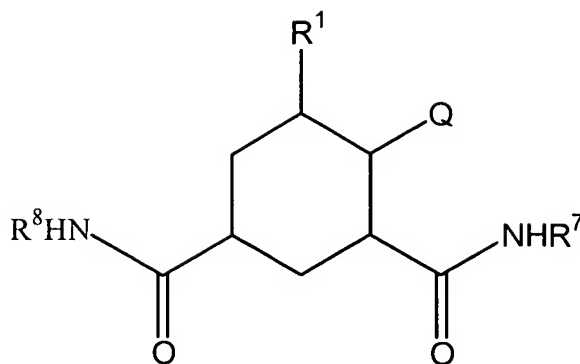
18. (Withdrawn) A chemical entity according to claim 17 which interferes with Site 1 binding of CAD of HIF to FIH and which exploits electrostatic, hydrogen binding and/or hydrophobic interactions with one or more residues selected from 102, 104, 106, 201, 202, 147, 239, 299-303, 313, 317, 318, 321, 324, 238, 296 or 321 to 324 of FIH.

19. (Withdrawn) A chemical entity according to claim 17 wherein said chemical entity interferes with binding of CAD of HIF to FIH at Site 2, and exploits electrostatic, hydrogen binding and/or hydrophobic interactions with residues 149, 150, 151, 152, 159, 162, 163, 167, 181, 182, 183, 184 or 185.

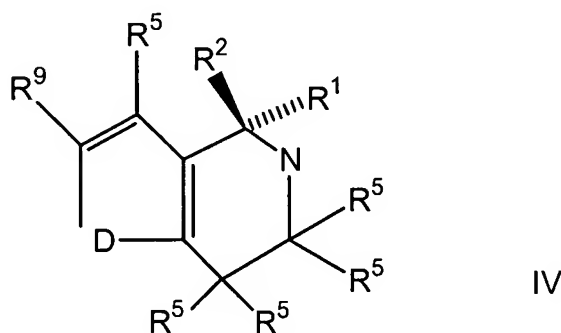
20. (Withdrawn) A chemical entity according to claim 17 wherein said chemical entity is a compound of the formula



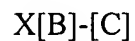
21. (Withdrawn) A chemical entity according to claim 17, wherein said chemical entity is a compound of the formula



22. (Withdrawn) A chemical entity according to claim 17 wherein said chemical entity is a compound of the formula

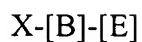


23. (Withdrawn) A chemical entity according to claim 17 wherein said chemical entity is a compound of the formula



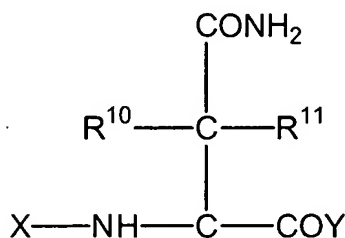
where X is as defined above, B is a linker group and C is an entity binding to part of the CAD binding site of FIH;

24. (Withdrawn) A chemical entity according to claim 17 wherein said chemical entity is a compound of the formula



where X and B are as defined above and E is an entity binding to part of the CAD when bonded to HIF.

25. (Withdrawn) A chemical entity according to claim 7 wherein said chemical entity is a compound of the formula



wherein X represents a valine residue or an analogue thereof and Y represents an alanine residue or an analogue thereof, R^{10} is fluorine or C_1 - C_3 alkyl, and R^{11} is fluorine, C_1 - C_3 alkyl or hydrogen or a corresponding compound R^{11} is absent or R^{10} and R^{11} form a methylene group.

26. Canceled

27. (Withdrawn) A method of treatment of a condition associated with increased or decreased HIF levels or activity or the treatment of a condition where it is desired to modulate HIF activity, which comprises administering to a patient a chemical entity according to claim 7.

28. (Withdrawn) A method according to claim 27 wherein said condition is ischaemia, wound healing, auto-, allo- or xeno-transplantation, systemic high blood pressure, cancer or an inflammatory disorder.

29. (Currently Amended) A method of identifying, screening, characterizing or designing a chemical entity which mimics or binds to a FIH (Factor Inhibiting HIF (Hypoxia Inducible Factor)), ~~which method comprises using the structural coordinates obtainable by~~ subjecting a crystal comprising FIH to X-ray diffraction measurements and deducing the structural coordinates from the diffraction measurements polypeptide comprising SEQ ID NO:21, or a fragment or mutant thereof, that adopts a similar 3-dimensional structure as described by the structural factors or structural coordinates shown in Table 3, and wherein the fragment or mutant retains asparaginyl hydroxylase activity, which method comprises using the structural coordinates shown in Table 3 and identifying, screening, characterizing or designing said chemical entity that mimics or binds to said FIH.

30. (Canceled).

31. (Previously Presented) A method according to claim 29, wherein said chemical entity binds to the FIH.

32. (Previously Presented) A method according to claim 29, wherein said chemical entity is selected to inhibit the asparaginyl hydroxylase activity of the FIH.

33. (Currently Amended) A method according to claim 29, further comprising contacting said chemical entity with a HIF polypeptide comprising SEQ ID NO:24 or 25 or a fragment thereof or variant having at least 90% identity thereof, wherein said fragment or variant retains the asparagine residue therein and retains the capacity to bind FIH, and with the FIH polypeptide or fragment or mutant thereof, and monitoring for hydroxylation of said asparagine residue ~~or a fragment thereof or a homologue of either thereof incorporating asparagine 803 with FIH or a homologue thereof which maintains the asparaginyl hydroxylase activity of FIH and monitoring for hydroxylation of asparagine 803.~~